TOXICOLOGICAL EXPERIMENTS WITH SOME OF THE HIGHER FUNGI

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INTRODUCTION

From the earliest times it has been observed that the consumption of certain species of mushrooms is followed by extremely unpleasant and occasionally fatal results. *Amanita muscaria* and *Amanita phalloides,* among others, have earned a very unsavory reputation by their ever growing list of fatalities. The danger from these plants is so great that usually only novices are unaware of their appearance and properties. Consequently, their death-roll receives its greatest additions from among foreigners and children. However, cases of mistaken identity have occurred even in the baskets of experienced persons. Among the species of *Amanita* we find many of the most poisonous forms, but other groups have dangerous representatives as well, although none quite so fatal.

The evil reputation of *Amanita muscaria* induced Schmiedeberg and Koppe* to investigate its poison from chemical and pharmacological standpoints. From their careful work it became evident that this plant contained an active principle which they called muscarin. This was at first considered an alkaloid of the general nature of strychnin and morphin, but later work has shown that it is probably a complex ammonia derivative. Muscarin is an extremely active substance and although present in the fungus in small amounts, it is still able to show its characteristic and fatal effects. Muscarin is particularly violent in its action on the nervous system, causing increased secretion, rapid pulse, then paralysis and finally cessation of heart action by stimulating the inhibitory nerve-endings of that organ. All of these effects may be neutralized by the administration of atropin in small doses; the latter being a complete antidote for pure muscarin. Unfortunately,

* Schmiedeberg and Koppe: Das Muskarin. Leipzig, 1869.

however, atropin cannot wholly prevent the harmful effects caused by eating *Amanita muscaria,* possibly because there are other toxic substances present in the plant. Harmsen† held this opinion as a result of his study of this fungus. He found that atropin was not a complete antidote for extracts of *Amanita muscaria,* and furthermore, that weight for weight his preparations from the fresh plant were twice as toxic as pure muscarin. From his experiments upon cats and dogs he calculated that if muscarin *alone* were responsible for the toxic effects of this plant, it would be necessary for a man of average weight to eat *four kilograms* of the fresh fungus in order to receive the lethal dose of pure muscarin. Therefore he postulated the existence of another poison in *Amanita muscaria,* calling it "Pilz-toxin." He claimed that this substance, when separated from muscarin in extracts of the fungus, was not neutralized by atropin, and produced long-continued convulsions and ultimate death. The work of Harmsen upon his "Pilz-toxin" has never been confirmed, but most of the evidence, clinical and otherwise, indicates that muscarin may not be the sole factor involved in cases of poisoning by *Amanita muscaria.* Ford* has also shown that in this species there are present peculiar substances that first cause an agglutination and finally a solution of the red corpuscles of the blood. However, muscarin is probably the toxic substance of greatest importance in *Amanita muscaria* because it withstands heating, whereas the associated materials which affect the blood as above stated, are destroyed by heat, and thus are prevented from acting after the ingestion of the cooked fungi.

Amanita phalloides is even more dangerous than *Amanita muscaria* because there is no known antidote for its poisonous principle. Several investigators have studied the poisons of this plant, but Ford† alone seems to have been able to isolate and learn the properties of its poisonous substances. The results of poisoning

† Harmsen: Zur Toxicologie des Fliegenschwammes. Archiv. f. Expt. Path. u. Pharmacologie. 1906, 1, p. 361.

* Ford: Distribution of poisons in the Amanitas. Jour. Pharmacol. and Expt. Therapeutics. 1909, i, p. 275.

† Ford: Distribution of haemolysins, agglutinins and poisons in fungi, especially the Amanitas, Entolomas, Lactarius and the Inocybes. Jour. Pharmacol. and Expt. Therapeutics. 1911, ii, p. 285. This paper has a complete bibliography.

by *Amanita phalloides* are distinct from those seen in the case of *Amanita muscaria,* since the latter apparently causes death by its action upon the nervous system. These effects are more serious than any caused by the blood-destroying substances found in so many mushrooms. Fortunately, however, the latter are not so important, owing to the ease with which they are destroyed by heat and the digestive juices. Autopsies after fatal *Amanita phalloides* poisoning of people and animals show that most of the internal organs are congested, hemorrhagic, and very seriously affected with necrosis and degeneration. In serious cases death intervenes in a few days, while muscarin poisoning develops in a few hours and runs rapidly to death or complete recovery in a short time. There is no antidote for poisoning by the so-called "amanita-toxin" of *Amanita phalloides,* nor is a rapid recovery to be expected, in view of the grave lesions it causes. As in the case of muscarin, the "amanita-toxin" is not destroyed by cooking. The blood-laking poisons of this same fungus are destroyed by heat and so probably they are always without effect unless the fungus is eaten in the raw state. Schlesinger and Ford* purified the "amanita-toxin" by rigorous chemical methods and obtained final products showing all the characteristic effects of the plant extracts which had been heated to destroy blood-laking substances. They found that the "amanita-toxin" did not seem to belong to the ordinary classes of the powerful poisons, such as the toxalbumins, alkaloids, etc.

Very recently Ford† has reported that in *Inocybe infelix* he found a peculiar poison that resisted heat and drying. In animals it did not produce the effects of muscarin, "amanita-toxin," or of any known mushroom poison. The symptoms came on at once and by their nature seemed to indicate the action of some powerful narcotic poison upon the nervous system. The most striking symptoms were extreme drowsiness, forcible retraction of the head (in rabbits), and complete paralysis lasting several hours. The smaller animals died but the larger ones recovered completely in a few hours. All of these observations seemed to indi-

* Schlesinger and Ford: On the chemical properties of Amanita-toxin. Jour. Biol. Chem., 1907, iii, p. 279.

† Ford: see footnote, page 176.

cate an active narcotic poison of a somewhat unique character. The fact that this *Inocybe* is very closely related to *Inocybe infida* makes Ford's observations very interesting in connection with our own upon this plant, as stated below.

A CASE OF POISONING BY INOCYBE INFIDA

The details of the poisoning by *Inocybe infida* of Dr. W. C. Deming and his family in this vicinity have already been published,* but they will be repeated here for the sake of completeness. We quote from Dr. Deming's own notes made at the time:

"I here transcribe notes made on that or the following evening: June 14, '09, about 11:30 A.M., my son and I gathered about a quart of mushrooms, mostly of the unknown variety and some of

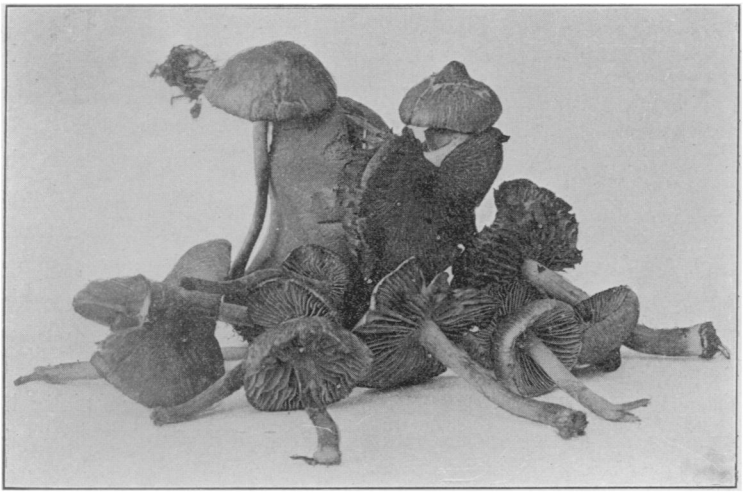

Fig. 1. *Inocybe infida* (Peck) Earle.

the variety frequently eaten. No other kind was gathered. These were stewed and served on toast at 1 P.M. I ate only one half slice with the mushroom thereon, some bread and butter, two cups of weak tea, a little more than one half a stuffed egg, with lettuce and mayonnaise dressing.

"Directly after lunch I smoked one half a cigarette as usual. On finishing this, I began to wonder if this or the mushroom had

* Murrill: A New Poisonous Mushroom. Mycologia, 1909, i, p. 211.

disagreed with me, on account of a slight 'queer' feeling which I cannot accurately describe, but it was so little at first that I dismissed it from my mind. In a few minutes, however, I gradually began to get a fullness in the head and a rapid heart action as if I had taken nitroglycerin. Then I began to sweat, with a feeling of heat over the body, so that my clothing was drenched, even my outer clothing requiring changing later. At the same time there was no nausea nor prostration nor other bad feeling, and I attended to a man with a wound in my office and then to other members of the family without difficulty, though a little confused in mind perhaps. A little after that, perhaps forty-five minutes after eating the mushroom, I washed out my stomach with a tube, and later took about an ounce of castor oil. Soon after, but long before the oil operated, I had a disagreeable sense of pressure, almost pain in the lower bowel, accompanied for a little while by slight abdominal soreness or pain. All symptoms gradually subsided and by evening I was as well as ever except for a little feeling of exhaustion.

"My wife, 25 years old, ate one whole slice of toast with mushrooms, two half eggs stuffed with lettuce and mayonnaise, tea, bread and butter. About half an hour later she felt nauseated and dizzy and lay down. I gave her five glasses of warm water, after which she vomited the egg, but saw no mushrooms. She then took castor oil.

"Mrs. A., 65 years old, ate the same amount of mushrooms, several slices of bread and butter, a cup of tea, but no eggs nor salad. When asked, said she felt slight indigestion, but otherwise well.

"My son, 5 years old, ate same amount, but no eggs nor salad. Immediately after lunch he had a diarrheal movement containing mushrooms. He was given ipecac and warm water and vomited some mushrooms.

"Sophie, maid, aged 30, tasted mushrooms. Felt nauseated soon after. Given mustard and water, but did not vomit. Later, castor oil and was purged and somewhat prostrated. Hattie, maid, aged 38, tasted mushrooms. Belched gas soon after. Not sick. Esther, maid, aged 24, tasted; no effects.

"There was no peculiar taste to the cooked mushrooms, perhaps

a very evanescent bitterness in the raw state. I thought perhaps the combination of the eggs and mayonnaise with the mushrooms had something to do with the effects, as my wife and I, the only ones who ate both in any amount, were the chief sufferers. In my case the beating of the heart, full head and sweating were very marked, though I ate but half as much as the others."

In this case we are fortunate in having a physician's careful description of the symptoms following the meal of harmful mushrooms. It should be noted that these symptoms were caused by the cooked fungi. That fact, taken with the nature of the symptoms, rapidity of recovery, etc., would indicate a toxic substance having more the nature of muscarin than that of blood-laking substances or of the "amanita-toxin," which, under analogous conditions, acts slowly.

EXPERIMENTAL

THE CHEMICAL METHODS OF THE INVESTIGATION

The general features of the clinical data in this case of poisoning seemed to indicate the action of an alkaloid. In some preliminary experiments it was found that the toxic principle could be extracted by hot or cold 95 per cent. alcohol, and that the evaporation residue from such toxic extracts, after being dissolved in water, yielded a slight yellowish precipitate with potassio-mercuric iodide (Mayer's reagent). We then applied to the available specimens of *Inocybe infida* the method of Harmsen* for the preparation of muscarin from *Amanita muscaria*. The air-dry plants of *Inocybe infida* are very small, those of average size usually weighing from 0.1 gram to 0.3 gram. The dry plants were powdered in a coffee-mill and treated as follows:

The powder was extracted twice for twenty-four hour periods with ten times its weight of 95 per cent. alcohol. The extraction was carried out at room-temperature with an occasional thorough shaking. The alcoholic solutions were evaporated to the consistency of a thick syrup on a water-bath. The syrup was extracted with a small volume (15–25 c.c.) of 95 per cent. alcohol. This extract was also evaporated to the consistency of a syrup,

* Harmsen: see footnote, page 176. Slight modifications of the method were introduced.

which, in turn, was thoroughly triturated with powdered glass until a stiff paste was formed. This paste was spread thinly on large watch-glasses and kept in a vacuum desiccator over sulphuric acid for a week. The desiccator was frequently exhausted.

The resultant dry friable mass was then extracted with three successive small portions of absolute alcohol. These solutions were combined, evaporated to dryness on a water-bath, treated with a small volume of water and filtered free from the large amount of fatty matter which separated out. The filtrate was usually clear and colorless, and neutral or slightly alkaline to litmus. When 40 grams of material were used, the volume of the final aqueous solution was about ten cubic centimeters. By evaporation, this volume of solution yielded from 0.05–0.15 gram of a semi-crystalline residue.

The modified method just outlined was adopted after several other variations of it had been tried. For instance, Harmsen used boiling alcohol to extract his material, but in our hands it was not as satisfactory as the cold alcohol, because the hot solvent dissolved a larger amount of gummy matter, and besides, such residues showed no greater toxicity than those obtained by extraction with cold alcohol. The success of this extraction method depends upon the repeated purifications that result from re-solution of the syrupy evaporation residues with fresh alcohol, and also upon the complete drying of the powdered-glass-syrup mixture in the desiccator. When the residue from the absolute alcohol extract was treated with water, a bulky insoluble portion was separated. The small amount of aqueous filtrate was used for injection into frogs.

The fact already mentioned, that potassio-mercuric iodide precipitated yellowish material when added to the *Inocybe* extract, led to the use of this reagent for the purification of the toxic substance. The method of alkaloid purification, as finally adopted, was that recommended by Dragendorff,* which was conducted as follows:

The aqueous filtrate obtained in the last phase of the Harmsen

* Dragendorff: Plant Analysis, translated by Greenish. London, 1884, pp. 57–8.

process was made slightly acid with sulphuric acid, and then treated with a moderate excess of potassio-mercuric iodide solution. A yellowish amorphous precipitate formed at once, and after heating the mixture for an hour on a water-bath, the precipitate was allowed to settle over night. Filtration through double filters was often necessary to remove the colloidal precipitate. The thoroughly washed precipitate was then suspended in hot water and decomposed with hydrogen sulphide, while the mixture was still hot. After this treatment the mercury sulphide could be filtered off readily, especially if the mixture was first allowed to stand on a steam-bath for an hour or more. The filtrate contained some free hydriodic acid and also the compound of the toxic substance with hydriodic acid. The *careful* addition of silver sulphate, in the form of a saturated aqueous solution of that substance, until no further precipitate was obtained, followed by boiling for a short time, served to decompose and precipitate all iodine derivatives. The yellow silver iodide was then filtered off and the sulphuric acid in the filtrate removed by precipitation with an excess of barium carbonate. The clear filtrate, from the resultant barium sulphate plus the physical excess of barium carbonate, contained any alkaloidal substance that occurred in the specimens under examination. We evaporated this aqueous solution to 10–15 c.c. on a water-bath and used small portions of it in the pharmacological tests on frogs as indicated below.

Thinking that cholin, resulting from the decomposition of lecithins in the fungi, might be present with the toxic substance in the final filtrates, we tested all of the latter for cholin. Rosenheim's* periodide test offers a beautiful and characteristic means of detecting cholin. Practically all the extracts were found to contain it. For the detection of cholin one adds a drop of platinum chloride solution to the liquid to be tested, which may be only a few drops on a microscope slide. After allowing the water to evaporate, the feathery and prismatic colorless crystals of the cholin-platinum chloride and of the excess of platinum chloride may be easily detected under the microscope. When these crystals

* Rosenheim: New Tests for Choline in Physiological Fluids. Jour. of Physiol. 1905, xxxiii, pp. 220–4.

are treated with a drop or two of Lugol's solution,* the microscopical appearance gradually changes; the original crystals slowly disappear, the whole field becomes darker, and finally characteristic brown platelets come to view in large numbers. The cholin periodide crystals have a great similarity to the "haemin" crystals obtained in Teichmann's test for hemoglobin. Upon evaporation of the water, the crystalline plates lose their form and change into oily drops, which immediately resume their characteristic form when more Lugol's solution is added.

Before applying the extraction and alkaloidal separation methods to *Inocybe* we tested them upon samples of *Amanita muscaria* to determine the efficacy of our procedures. The methods were found to be satisfactory.

TOXICOLOGICAL EXPERIMENTS

In our experiments to determine the toxicity of extracts upon frogs we used lively animals weighing from 25 to 40 grams. All injections were made into the dorsal lymph-sac except in a few cases, when the toxic solution was given by mouth through a small narrow pipette. A description of these experiments follows.

EXPERIMENTS WITH AMANITA MUSCARIA. Twenty-five grams of air-dry specimens were treated and extracted as already described, but no attempt was made to apply the alkaloidal separation process. The final volume of the aqueous solution was 15 c.c., which on evaporation to dryness yielded 0.18 gram of a waxy, semicrystaline residue. This residue gave a striking Rosenheim cholin test.

Experiment 1. July 14, 1910. Frog 2. Weight, 27 grams. Received an injection of 1 c.c. of *Amanita muscaria* extract at 2.36 P.M.

2.38 P.M. Paralyzed after excitement.

2.41 P.M. Paralyzed, very slight muscular reflexes.

2.47 P.M. Heart stopped in diastole.

Experiment 2. July 14, 1910. Frog 3. Weight, 31 grams. At 9.58 A.M. received an injection of 0.5 c.c. of the extract administered to the frog in Experiment 1.

* Lugol: Lugol's solution contains 4 grams of iodine and 6 grams of potassium iodide dissolved in 100 c.c. of water.

10.02 A.M. Paralyzed.

10.15 A.M. Apparently recovered.

10.20 A.M. Paralyzed again.

10.27 A.M. Heart stopped in diastole.

The remainder of the available air-dry specimens (20 grams) was extracted according to Harmsen's scheme and the residue thus obtained was subjected to the alkaloidal separation process already outlined. A small amount of a waxy residue was the outcome of this treatment. This residue weighed 0.07 gram. It was dissolved in 10 c.c. of water. This solution also responded to the test for cholin.

Experiment 3. August 11, 1910. Frog 11. Weight, 29 grams. Received an injection of 1 c.c. of extract at 4.27 P.M.

4.31 P.M. Completely paralyzed.

4.35 P.M. Heart stopped.

EXPERIMENTS WITH INOCYBE INFIDA. FIRST GROUP. Twenty grams of pulverized *Inocybe infida* were extracted in the usual way and the final residue, weighing 0.21 gram, was dissolved in 10 c.c. of water. The residue was greenish and semi-crystalline. Its solution was slightly alkaline to litmus. This extract was not subjected to the alkaloidal separation process.

Experiment 4. July 15, 1910. Frog 8. Weight, 35 grams. Received an injection of 1 c.c. of *Inocybe* extract at 2.30 P.M.

2.37 P.M. Lethargic. Swallowing motions.

2.55 P.M. Lethargic. Swallowing motions. Began to appear bloated. July 16, 10.30 A.M. Heart stopped. Animal bloated.

Experiment 5. July 15, 1910. Frog 9. Weight, 26 grams. At 3.56 P.M. received an injection of 2 c.c. of the extract administered to the frog in Experiment 4.

4.01 P.M. Nearly paralyzed.

4.17 P.M. Wholly paralyzed.

4.21 P.M. Heart stopped.

Forty grams of pulverized *Inocybe infida* were extracted and treated in the usual manner. The residue, which was waxy and greenish in color, weighed 0.36 gram. It was dissolved in 20 c.c. of water. This solution contained cholin, as was indicated by the periodide test. The alkaloidal separation process was not applied to it.

Experiment 6. July 26, 1910. Frog 10. Weight, 31 grams. Received an injection of 1 c.c. of this extract at 2.55 P.M.

3.15 P.M. Lethargic.

5.30 P.M. Partly paralyzed.

July 27, 9.30 A.M. Apparently normal.

Experiment 7. July 28, 1910. Frog 10a. Weight, 28 grams. At 2.06 P.M. received an injection of 1.5 c.c. of the extract administered to the frog in Experiment 6.

2.12 P.M. Partly paralyzed.

3.30 P.M. Wholly paralyzed.

July 29, 9.20 A.M. Apparently normal.

Experiments 6 and 7 were repeated in every particular, with a new extract. The final residue weighed 0.43 gram. It was dissolved in 20 c.c. of water. The test for cholin was positive.

Experiment 8. January 20, 1911. Frog 16. Weight, 33 grams. Received an injection of 1 c.c. of this extract at 3.12 P.M.

3.16 P.M. Excited.

3.23 P.M. Very lethargic, partly paralyzed.

3.30 P.M. Wholly paralyzed.

4.00 P.M. Recovering. Bloated. Partly paralyzed.

4.30 P.M. Slightly paralyzed.

January 21, 9.10 A.M. Lethargic(?), bloated.

4.00 P.M. Normal, bloated(?).

Experiment 9. January 19, 1911. Frog 17. Weight, 37 grams. At 4.07 P.M. received an injection of 1 c.c. of the extract administered to the frog in Experiment 8.

4.13 P.M. Excited.

4.25 P.M. Very lethargic.

4.40 P.M. Lethargic and partly paralyzed.

January 20, 9.00 A.M. Lethargic and bloated.

January 21, 9.15 A.M. Normal.

SECOND GROUP. The evaporated remainders of the *Inocybe* extracts prepared for use in Experiments 4–9 were combined, redissolved, and the alkaloidal separation process applied to the resulting solution. The precipitate given with potassio-mercuric iodide was light in color and in such a finely divided state that it was filtered off with difficulty. The precipitate was not as yellow as that from *Amanita muscaria,* nor was it as crystalline in ap-

pearance. The final residue was greenish and weighed 0.13 gram The test for cholin was positive. The solution was neutral to litmus.

Experiment 10. February 13, 1911. Frog 18. Weight, 24 grams. Received an injection of 1 c.c. at 2.07 P.M.

2.25 P.M. Excited.

2.32 P.M. Partly paralyzed.

2.35 P.M. Completely paralyzed.

4.30 P.M. Lethargic and partly paralyzed.

February 14, 9.15 A.M. Apparently normal.

EXPERIMENTS WITH CLITOCYBE MULTICEPS. Having already studied the action of extracts of the poisonous *Amanita muscaria* and the questionable *Inocybe infida,* we performed similar experiments with comparable extracts of *Clitocybe multiceps,* which is known to be harmless. This plan was intended to serve as a "control" of our previous procedure and to show whether we had introduced poisonous material into our extracts during their preparation. About 50 grams of dried *Clitocybe multiceps* were treated in the usual manner and finally 0.23 gram of a waxy residue was obtained. This residue was dissolved in 10 c.c. of water. The solution seemed to be slightly alkaline to litmus. The test for cholin was very pronounced, in fact, it was the most striking of any yet observed by us in this study.

Experiment 11. October 14, 1910. Frog 13. Weight, 35 grams. Received an injection of 1 c.c. of the extract at 9.47 A.M.

10 A.M. No symptoms.

12 M. Normal and continued so.

Experiment 12. October 14, 1910. Frog 14. Weight, 25 grams. At 10.50 A.M. received an injection of 2 c.c. of the extract administered to the frog in Experiment 11.

11.25 A.M. No symptoms.

12.00 M. Slightly lethargic(?).

12.45 P.M. Normal.

October 15, 9.10 A.M. Normal and continued so.

The remainder of the extract prepared for use in Experiments 11 and 12 was treated according to Dragendorff's method for the separation of alkaloids. The resultant residue weighed 0.05 gram. It was dissolved in 10 c.c. of water. The solution was slightly

alkaline to litmus. Apparently there was no cholin in it, for there was no response to the Rosenheim test.

Experiment 13. October 24, 1910. Frog 15. Weight, 28 grams. Received an injection of 1 c.c. of the extract at 4.07 P.M.

4.17 P.M. Normal.

5.05 P.M. Normal.

October 25, 9.05 A.M. Normal.

Experiment 14. October 28, 1910. Frog 13a. Weight, 32 grams. At 2.28 P.M. received an injection of 1 c.c. of the extract administered to the frog in Experiment 13.

3.02 P.M. Normal.

3.25 P.M. Normal.

4.50 P.M. Normal.

Experiment 15. October 28, 1910. Frog 20. Weight, 35 grams. At 4.50 P.M. received, *per os,* 1 c.c. of the extract administered to the frog in Experiments 13 and 14.

5.05 P.M. Excited(?).

5.20 P.M. Normal.

5.50 P.M. Normal.

SUMMARY OF CONCLUSIONS

Inocybe infida, when subjected to processes of extraction and purification that separate muscarin from *Amanita muscaria,* yields material which exerts definite toxic effects upon frogs. These effects are quite different from those produced by muscarin as obtained, by the same method, from *Amanita muscaria.* A prolonged state of lethargy, often with complete recovery after twelve or fifteen hours in this condition, was a constant factor in our toxicological experiments with this *Inocybe.* The poison seems to be of the narcotic type recently found by Ford in *Inocybe infelix.*

The poison of *Inocybe infida* seems to belong chemically to the class of alkaloids or related substances. The plants of this species are small. A very much larger supply of these mushrooms than the available quantity will be required for the isolation and chemical identification of the toxic material.

Dr. William A. Murrill called Professor Gies' attention to the

case of poisoning in Dr. Deming's family (p. 178) and suggested the desirability of an inquiry into the cause. At Professor Gies' request we conducted these experiments. The sincere thanks of the writers are due to Professor Gies for his interest and suggestions. Dr. Murrill very kindly supplied us with the fungi used in this work, and to him also our thanks are due.

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